

Studies of the medicinal plant *Euphorbia hirta* methanol leaf extract phytochemicals by GCMS analysis.

Igwe K. K.¹, Madubuike A.J.¹, Akomas S.C.¹, Otuokere I. E.² Ukwueze C. S.³

¹Department of Veterinary Physiology, Pharmacology and Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria.

²Department of Chemistry, Michael Okpara University of Agriculture, Umudike, Nigeria.

³Department of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria.

Corresponding Author: kkgwe191@gmail.com

ABSTRACT: Phytochemicals in methanolic extract of *Euphorbia hirta*, leaf was studied using GC MS analysis. Ten compounds were identified from the extract. The major chemical constituents were Niacin or Nicotinic acid [Peak area: 31.70% ; RT: 22.718; Mol formula: $C_6H_5NO_2$], S-methyl-L-cysteine [Peak area: 18.88%; RT: 21.794; Mol formula: $C_4H_9NO_2S$], Methyl 1,4-methylpentadecanoate [Peak area :11.22% ; RT: 19.326; Mol formula: $C_{17}H_{34}O_2$], 2-amino-3-sulfanypropanoic acid [Peak area: 5.16%; RT: 21.682; Mol formula: $C_3H_7NO_2S$], 4-amino-4-oxobut-2-enoic acid [Peak area: 4.02%; RT: 23.118; Mol formula: $C_4H_5NO_3$]. The bioactive compounds in the methanol leaf extract of *Euphorbia hirta*, exhibited phytopharmacological significance and hence could be beneficial for therapeutic use against some health challenges.

Keywords: GCMS, *Euphorbia hirta*, Asthma plant, Hallucination; Nicotinic acid.

I. INTRODUCTION

Euphorbia hirta is an annual hairy plant with many stems and branches from the base to top that is reddish or purplish in colour [1]. It belongs to the plant family Euphorbiaceae. It is a pantropical weed, known to be native in India. It is found commonly in grassfields in Ohafia town in Nigeria. It grows in open grasslands, road sides and pathways and widely used as a medicinal herb [2]. Its common names are asthma plant, hairy spurge [3]. It is an annual herb that grows erect with a hairy solid stem that produces white latex or milky juice in abundance, which can be used as arrow poisons [4]. It has simple leaves that are elliptical and hairy on both surfaces. The leaves grow as opposite pairs on the stem and flowers are found on each leaf node. The fruit occurs in capsules producing tiny, oblong, four-sided red seeds. Other species of *Euphorbia* are useful in traditional medicines with *Euphorbia hirta* possessing pharmacological properties against bacterial activity like *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* [5], anthelmintic effect, antioxidant effect [6] antiasthmatic and sedative effect, antispasmodic, antifertility effect [7], anti inflammatory effect [8, 9], antifungal activity against plant pathogens *Colletotrichum capsici*, *Fusarium pallidroseum*, *Botryodiplodia theobromae*, *Phomopsis scaricae-papayae*, and *Aspergillus niger* using the paper disc diffusion technique [10] and antimalarial properties [11]. It is widely used as a medicinal plant and commonly called asthma plant or asthma herb [11]. Pill bearing spurge, garden spurge or pill pod sandran in English [3]. In the milky latex of *Euphorbia hirta* there are also some phytochemicals like sterols, alkaloids, tannins, quercetin, glycosides, triterpenoids, alkenes phenolic acids, choline and shikimic acid [12]. It is traditionally used to treat conjunctivitis ulcerated cornea, asthmatic bronchitis, intestinal disorders, syphilis and to increase the milk flow in lactating women [13]. The aqueous extract of this plant exhibits analgesic, anti pyretic and anti-inflammatory activities which can be used in the treatment of swellings, boils, eyelid stress, and cancer growth [13]. The methanolic extract of the leaves have antifungal and antibacterial activities. Roots are also used for snake bites. The ethanol extract of the whole plant showed hypoglycaemic activity in rats and also a sedative effect on the genitor-urinary tract [2, 13, 14]. The present research was carried out to determine the phytochemical components in *Euphorbia hirta* by GC-MS analysis. Recently, interest for the characterization of organic compounds in plants has increased. Therefore, the research is to screen and isolate the bioactive compounds, evaluate the pharmacological potentials and characterize them by GC-MS analysis.

II. MATERIALS AND METHODS

A Plant Materials

Fresh leaves of *Euphorbia hirta* was harvested at Ohafia town in Abia State, Nigeria. The plant leaves were identified by Prof M C Dike at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.



Figure 1 shows picture of *Euphorbia hirta* harvested in Ohafia town in Nigeria

B Preparation of Plant Extract

The plant material of *Euphorbia hirta* was collected from open grassland, shade dried for 10 days and pulverized to powder using mechanical grinder. The plant extract was prepared using Soxhlet method as described by [15]. Thirty grams (30 g) of powdered sample was introduced into the extraction chamber of the Soxhlet extractor using methanol as solvent. Temperature was maintained at 75° C throughout the extraction period of 48 hrs. At the end of the extraction period, the extract was concentrated using oven at 35° C to obtain dried extract which was sent for GCMS analysis.

C GCMS analysis of Euphorbia hirta

The characterization of the Phytochemicals in *Euphorbia hirta* was done using GC-MS QP2010 Plus (Shimadzu, Japan). The identification of the phytochemicals in the sample was carried out using a QP2010 gas chromatography with Thermal Desorption System, TD 20 coupled with Mass Spectroscopy (Shimadzu). The ionization voltage was 70eV. Gas Chromatography was conducted in the temperature programming mode with a Restek column (0.25 mm, 60 m, XTI-5). The initial column temperature was 80°C for 1min, and then increased linearly at 70°C min⁻¹ to 220°C, held for 3 min followed by linear increased temperature 10°C min⁻¹ to 290°C for 10 min. The temperature of the injection port was 290°C and the GC-MS interface was maintained at 290°C. The sample was introduced via an all-glass injector working in the split mode, with helium carrier gas low rate of 1.2 ml min⁻¹. The identification of compounds was accomplished by comparison of retention time, peak area percentage and fragmentation pattern, as well as with mass spectra of the GC-MS.

D Identification of Phytochemicals in Euphorbia hirta

GC-MS Chromatogram of *Euphorbia hirta* revealed eight peaks showing that eight different compounds were present. Identity of the active components in the extract was done by comparison of their retention indices, peak area percentage and mass spectra fragmentation pattern with those stored in the database of National Institute of Standards and Technology (NIST) and also with published literature, NIST08.LIB [16], WILEY8.LIB [17], PESTEI-3.LIB and FA-ME.LIB library sources were used for matching the identified components with the plant material. From there, the name, molecular weight, formula, structure and bioactivities of the compounds were ascertained.

III. RESULTS AND DISCUSSION

A Results

GCMS chromatogram of the methanol extract of *Euphorbia hirta* (Figure 2) showed ten peaks which indicated the presence of ten major phytochemical constituents.

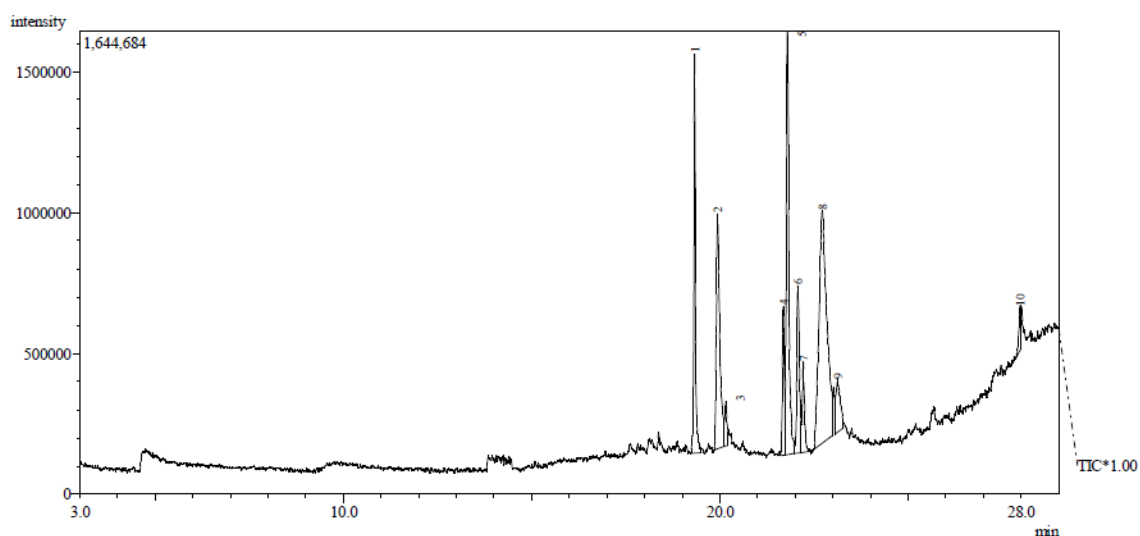
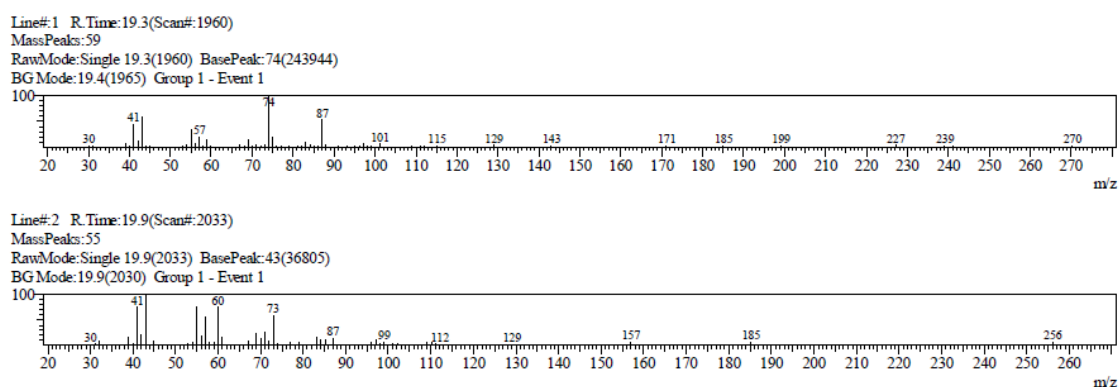


Figure.2 Showing the chromatogram of *Euphorbia hirta*,



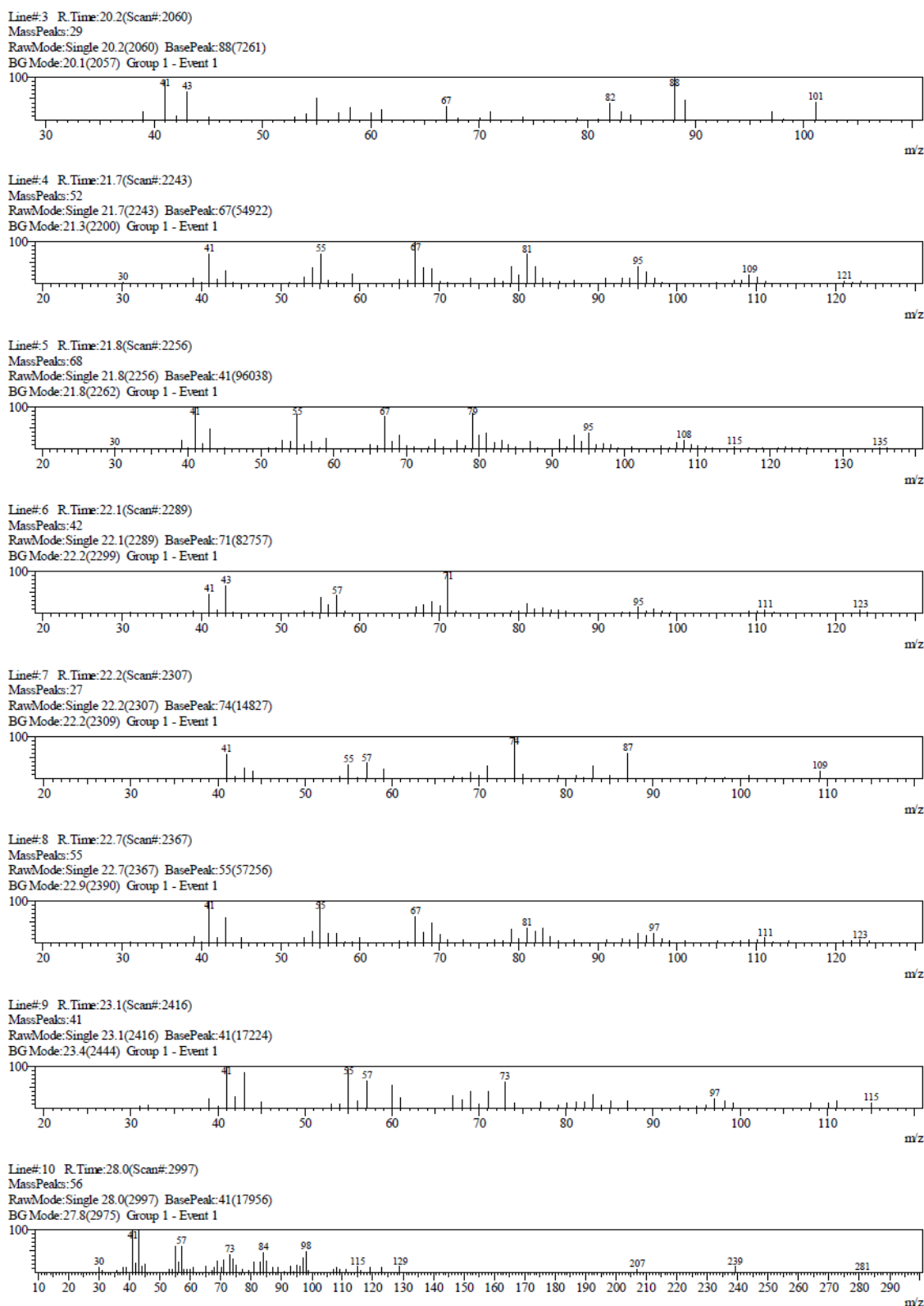
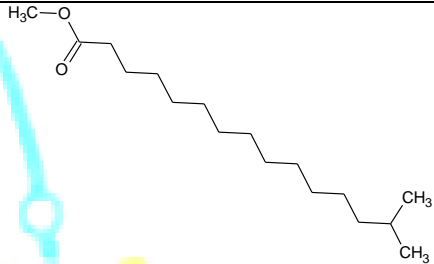
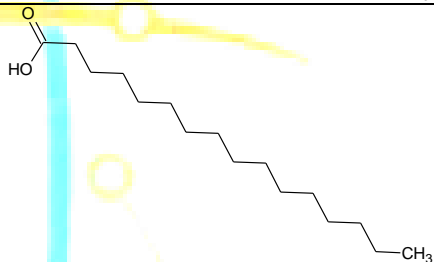

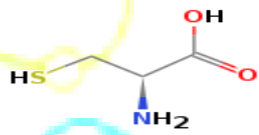
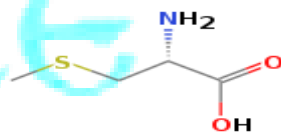
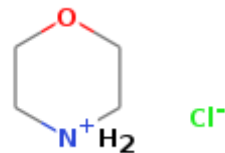
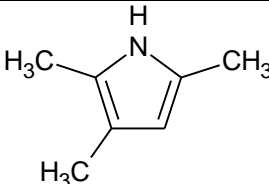
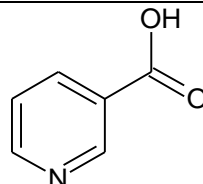
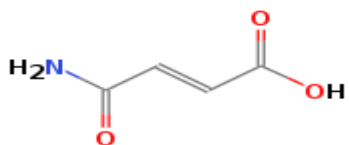
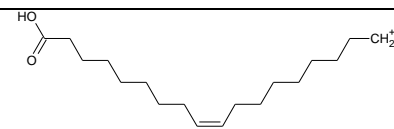


Figure 3 Shows the mass spectra of the ten phytochemicals identified by GCMS analysis in *Euphorbia hirta*,

Table 1. Name, retention time, peak area, mol. formula, mol. weight, structure and bioactivity of phytochemicals in methanol extracts of *Euphorbia hirta*,

S/No	Name of Compound	Retention time	Peak area %	Molecular weight	Molecular formula	Molecular structure	Bioactivity
1	Methyl 14-methylpentadecanoate	19.326	11.22	270.45	$C_{17}H_{34}O_2$		Catechol-O-Methyl-Transferase-Inhibitor and Methyl-Guanidine-Inhibitor
2	Hexadecanoic acid also known as Palmitic acid	19.937	4.12	256.42	$C_{16}H_{32}O_2$		Acidifier, Acidulant, Arachidonic acid, Arachidonic-Acid-Inhibitor, increase Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric Acid
3	5-methyl-1,3-oxazolidin-2-one	20.157	1.88	101.10	$C_4H_7NO_2$		Catechol-O-Methyltransferase-Inhibitor, Methyl-Donor, Methyl-Guanidine-Inhibitor
4	2-amino-3-sulfanylpropanoic acid	21.682	5.16	121.15	$C_3H_7NO_2S$		Increase Aromatic Amino Acid Decarboxylase Activity, Arachidonic acid-Inhibitor, Inhibit Production of Uric Acid
5	S-Methyl-L-cysteine	21.794	18.88	135.18	$C_4H_9NO_2S$		Low Sodium, Catechol-O-Methyl-Transferase-Inhibitor, Catechol-O-Methyltransferase-Inhibitor, Methyl-Guanidine-Inhibitor, 12-Lipoxygenase-Inhibitor, 5-Lipoxygenase-Inhibitor, Anti-LDL, Anticancer (Liver), Anticancer (Lung), Benzodiazepine-Receptor Ligand

Studies of the medicinal plant Euphorbia hirta methanol leaf extract.....

6	Chloromorpholin-4-ium	22.068	7.83	123.58	C ₄ H ₁₀ ClNO		Not found
7	2,3,5-trimethyl-1 <i>H</i> -pyrrole	22.215	3.65	109.16	C ₇ H ₁₁ N		11B-HSD-Inhibitor, 17-beta-hydroxysteroid dehydrogenase-Inhibitor, 5-HETE-Inhibitor, 5-HT-Inhibitor, 8-HETE-Inhibitor, Anti-5-HT, Anti-HIV-Integrase, Antidote (Heavy Metals), Hallucinogen, Aryl-Hydrocarbon-Hydroxylase-Inhibitor
8	Niacin or nicotinic acid	22.718	31.70	123.10	C ₆ H ₅ NO ₂		Anticancer (Oral), Antidote (organo-P), Antidote (Organophosphorus), Orexigen
9	4-amino-4-oxobut-2-enoic acid	23.118	4.02	115.08	C ₄ H ₅ NO ₃		Acidifier, Acidulant, Arachidonic acid, Arachidonic-Acid-Inhibitor, ncrease Aromatic Amino Acid Decarboxylase Activity, Inhibit Production of Uric Acid
10	17-carboxyheptadec-9-en-1-ylum	27.975	1.55	281.45	C ₁₈ H ₃₃ O ₂		Decrease Endothelial Leukocyte Adhesion, Decrease Endothelial Platelet Adhesion, Encephalopathic, Endoanesthetic, Endocrinactive, Ergotamine-Enhancer, Enterotoxic, Enterotonic, Enteromotility-Enhancer, Enterodepressant, Enkephalinogenic, Energizer

B Discussion

The chromatogram of *Euphorbia hirta* showed ten peaks indicating the presence of ten major compounds. Shown in table 1, Catechol-O-Methyl Transferase (COMT) is an enzyme that catalyses the breakdown of catecholamine forming homovanillic acid, nor metanephrine and metanephrine depending on the substrate. [18]. The phytocompound methyl-4-methylpentadecanoate [RT: 19.326, Peak Area: 11.22%, Molecular formula: $C_{17}H_{34}O_2$] showed inhibitory activity against COMT which may lead to an accumulation of catecholamine. Methyl transferase is any enzyme that catalyzes the transfer of a methyl group from a donor to an acceptor. [19] Catecholamine is a group of sympathetic-mimetic amines (dopamine, adrenaline and nor adrenaline) with aromatic portion whose molecule is catechol. The release of catecholamine at sympathetic nerve ending increases the rate and force of muscular contraction of the heart, thereby increasing cardiac output, constricts peripheral blood vessels, resulting in elevated blood pressure and also promotes an increase in blood lipids by increasing the catabolism of fats. [20]. Arachidonic acid inhibition activity demonstrated by Hexadecanoic acid at [RT: 19.937, Peak area: 4.12%, Molecular formula: $C_{16}H_{32}O_2$] by GCMS screening shows that the compound could be useful in the regulation of prostaglandin, leukotrienes and thromboxane production. S-methyl-cysteine at [RT: 21.794, Peak Area: 18.88%, Molecular formula: $C_4H_9NO_2S$] was the second most abundant compound in the extract and showed 12-Lipoxygenase and 5-Lipoxygenase inhibition activity. It showed also anti-low density lipoprotein (anti-LDL). The phytocompound could therefore be useful in checking the production of LDL by inhibiting lipoxygenase, an enzyme involved in the synthetic pathway of arachidonic acid to leukotrienes and thromboxane. S-methyl-L-cysteine had activity of inhibiting 1,2-lipoxygenase, 5-lipoxygenase therefore can inhibit the production of thromboxane and leukotrienes. Lipoxygenase (LOX) is an enzyme found in the lungs, platelets and white blood cells; involved in the arachidonic acid cascade leading to the production of leukotrienes and lipoxins. [21]. The most abundant compound in the extract was niacin or nicotinic acid [RT: 22.718, Peak Area: 31.70%, Molecular formula: $C_6H_5NO_2$] which showed anticancer and antidote for organophosphates. Nicotinic acid being the highest at retention time of 22.718 and peak area of 31.70%, molecular formula $C_6H_5NO_2$ had bioactivity of reducing the risk of cardiovascular disease and for treating pellagra [22, 23]. One of the interesting things in this *Euphorbia hirta* extract is in the compound 4-amino-4-oxobut-2-enoic acid [RT: 23.118, Peak Area 4.02%, molecular formula $C_4H_5NO_3$] and 2-amino-3-sulfanypropanoic acid [RT: 21.682, Peak Area 5.16%, molecular formula $C_3H_7NO_2S$] could inhibit uric acid production, therefore could be useful in the control of gout. Inhibition of production of uric acid means control of gout. Gout is a disorder of uric acid metabolism in which there is hyperuricemia and deposition of urates in and around the joints leading to a severe pain. This condition which can only be controlled by allopurinol can now have a phytochemical remedy. Allopurinol is a structural analogue of purine that directly inhibits xanthine oxidase [24]. The inhibition of uric acid production by 2-amino-3-sulfanypropanoic acid may be as a result of inhibition of xanthine oxidase. The compound 4-amino-4-oxobut-2-enoic acid also showed increase in aromatic amino acid decarboxylase activity which is associated with increase production of useful amines, for example, decarboxylation of tyrosine will yield adrenaline while that of tryptophan will yield 5-hydroxytryptamine (Serotonin). The phytocompound 4-amino-4-oxobut-2-enoic acid showed a bioactivity of increase decarboxylase activity of the aromatic amino acids which may result in increased production of serotonin and adrenaline. Serotonin is an inhibitory neurotransmitter that plays a role in regulating pain sensory perception, eating, sleep and body temperature. It has also been related to hallucinations and psychosis. [25]. In times of acute stress, adrenaline is released into the blood. Adrenaline is an emergency hormone and an integral for fight, flight, and fright syndrome. The release of adrenaline speeds up coronary blood flow and heart rate among other effects; oxygen supply to the heart is diminished because adrenaline causes the bronchi to dilate; thus it could be used to treat asthma. [11]. Of the estimated half a million species of plants about 150 have been used for hallucinogenic purposes [26]. At retention time of 22.215 and Peak Area % of 3.65, the phytocompound 2,3,5-trimethyl-1-H-pyrole indicated hallucinating and anti-HIV integrase activity. Therefore the compound is capable of producing false sensory perceptions and can act by not allowing the integration of the HIV virus into the host cell.

IV. CONCLUSION

The results revealed that Phytoconpounds obtained from the *Euphorbia hirta* methanol leaf extract contain ten bioactive compounds in their various concentrations. The major components were Nicotinic acid 31.70%, followed by S-methyl-cysteine 18.88%, and Methy l4-methylpentadecanoate 11.22%.

V. Acknowledgment

We are grateful to National Research Institute for Chemical Technology [NARICT], Zaria, Nigeria, for running the GC-MS analysis and Mrs Rose Sangodare for her effort to safe guard the sample for accurate result.

References

- [1]. Williamson EM. China. Churchill Livingstone. *Major Herbs of Ayurveda*, 2002.
- [2]. Sood, S. K., Bhardwaj, R., Lakhanpal, T. N., Ethnic Indian Plants in cure of diabetes. *Scientific Publishers*, 2005.
- [3]. Kumar S, Malhotra R, Kumar D. *Euphorbia hirta*: Its chemistry, traditional and medicinal uses, and pharmacological activities. *Pharmacognosy*, 2010 Rev 4 (7): 58–61.
- [4]. Open Source for Weed Assessment in Lowland Paddy Fields (OSWALD). *Asia IT&C Programme of the European Union*. 2007-07-21. Retrieved August 30, 2013.
- [5]. Ogbulie J.N., Ogueke C.C., Okoli I.C., Anyanwu B.N., Antibacterial activities and toxicological potentials of crude ethanol extracts of *Euphorbia hirta*. *Afr J Biotechnol*. 2007;6:1544–8.
- [6]. Sharma N.K., Dey S., Prasad R., In vitro antioxidant potential evaluation of *Euphorbia hirta* L. *Pharmacologyonline*. 2007; 1:91–8.
- [7]. Mathur A., Dixit V.P., Dobal M.P., Antifertility plant product: *Euphorbia hirta* in males. Proceedings of the International Symposium on Male Contraception: Present and Future. 1995
- [8]. Martinez V., Mariano A., Teresa O.R., Lazcano M.E., Bye R. Anti-inflammatory active compounds from the n-hexane extract of *Euphorbia hirta*. *Rev Soc Quim Méx*. 1999; 43:103
- [9]. Lanhers M.C., Fleurentin J., Dorfman P., Mortier F., Pelt J.M., Analgesic, antipyretic and antiinflammatory properties of *Euphorbia hirta*. *Planta Med*. 1991; 57:225–31.
- [10]. Mohamed S., Saka S., EL-Sharkawy S.H., Ali A.M., Muid S., Antimycotic screening of 58 Malaysian plants against plant pathogens. *Pestic Sci*. 1996;47:259–64
- [11]. Raymond Goldberg. Drugs across the spectrum. *Fourth edition* U.S.A. 2003 pg102-103
- [12]. Vol. 3 New Delhi: Council of Industrial and Scientific Research. 2005. The Wealth of India (Raw material)
- [13]. Galvez J., Zarzuelo A., Crespo M. E., Lorente M. D., Ocete M. A., Jimenez, J. Antidiarrheal activity of *Euphorbia hirta* extract and isolation of an active flavanoid constituent. *Planta Med*. 1993; 59: pp 333 – 336
- [14]. Kirtikar, K. R., Basu, B.D Dehradun, India: Oriental Enterprises; 2003. Indian medicinal plants with illustrations
- [15]. Jensen W.B., The origin of Soxhlex Extraction. *Journal Clinical Education* , 2007, 9084 (12),1913-1914.
- [16]. Stein S. E., National Institute of Standards and Technology (NIST) Mass Spectral Database and Software, 1990, Version 3.02,USA.
- [17]. Mc Lafferty F. W., Registry of mass spectral data ,1986, *Fourth electronic ed*.Wiley New York.
- [18]. Stiles G.L., Caron M.G., Lefkowitz R.K., The beta-adrenergic regulator: Biochemical mechanisms of physiological regulation. *Physiol Rev* 1984; 64:661
- [19]. V.P. Studdert, C.C.Gay, D.C. Blood. Saunders Comprehensive Veterinary Dictionary 4th Edition, New York. 2012 pp 703
- [20]. V.P. Studdert, C.C.Gay, D.C. Blood. Saunders Comprehensive Veterinary Dictionary 4th Edition, New York. 2012 pp 192
- [21]. V.P. Studdert, C.C.Gay, D.C. Blood. Saunders Comprehensive Veterinary Dictionary 4th Edition, New York. 2012 pp 652
- [22]. Bruckert E., Labreuche J., Amarenco P., Meta-analysis of the effect of nicotinic acid alone or in combination on cardiovascular events and atherosclerosis. *Atherosclerosis* 2010, 210 (2): 353–61. [doi:10.1016/j.atherosclerosis.2009.12.023](https://doi.org/10.1016/j.atherosclerosis.2009.12.023). PMID 20079494.
- [23]. Duggal J.K., Singh M., Attri N., Singh P.P., Ahmed N., Pahwa S., Molnar J., Singh S., Khosla S., Arora R., Effect of niacin therapy on cardiovascular outcomes in patients with coronary artery disease. *Journal of cardiovascular pharmacology and therapeutics* 2010,15 (2): 158–66. [doi:10.1177/1074248410361337](https://doi.org/10.1177/1074248410361337). PMID 20208032.
- [24]. Pacher P., Nivorozhkin A., Szabó C., Therapeutic Effects of Xanthine Oxidase Inhibitors: Renaissance Half a Century after the Discovery of Allopurinol. *Pharmacological Reviews*. 2006, 58 (1): 87–114
- [25]. Leo P. W. Chiu, Differential diagnosis and management of hallucinations"(PDF). Journal of the Hong Kong Medical Association 1989 41 (3): 292–7
- [26]. R.E. Schultes, A.Hofmann. Plant of the Guts, Origins of Hallucinogenic Use (*New York: Mc Graw-Hill*, 1979)